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A conservation genomics workflow to guide practical management actions

Maurizio Rossetto [a, b, *], Jia-Yee Samantha Yap [a], Jedda Lemmon [c], David Bain [c], Jason Bragg [a], Patricia Hogbin [d], Rachael Gallagher [e], Susan Rutherford [f], Brett Summerell [g], Trevor C. Wilson [g]

[a] Research Centre for Ecosystem Resilience, Australian Institute of Botanical Science, The Royal Botanic Garden Sydney, Australia
[b] Queensland Alliance of Agriculture and Food Innovation, University of Queensland, Brisbane, Australia
[c] Ecosystems and Threatened Species, Biodiversity Conservation and Science, NSW Department of Planning Industry and Environment, Wollongong, Australia
[d] PO Box 694, Singleton, NSW, 2330, Australia
[e] Department of Biological Sciences, Macquarie University, North Ryde, NSW, 2109, Australia
[f] Institute of Environment and Ecology, School of the Environment and Safety Engineering, Jiangsu University, China
[g] Australian Institute of Botanical Science, Royal Botanic Gardens and Domain Trust, Sydney, Australia

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ABSTRACT

Owing to decreasing costs and increased efficiency, it is now conceivable that conservation genomic information can be used to improve the effectiveness of recovery programs for many, if not most, threatened plants. We suggest that a simple genomic study be viewed as an initial step in conservation decision-making, as it informs long-term recovery efforts in various ways. We present biodiversity managers and conservation biologists with a simple, standardized workflow for genomic research that can guide efficient collection, analysis and application of genomic information across disparate threatened plants. Using two case studies, '*Banksia vincentia*' and *Daphnandra johnsonii*, we demonstrate how a single round of genotyping by sequencing – a one-time cost – produces multiple directly applicable benefits, and how generating genomic information as early as possible can enhance conservation outcomes. We argue for a shift away from asking whether genomic information is needed or justified, and a shift towards consideration of the questions that need to be addressed. Such questions should aimed at cost-effectively guiding multiple practical aspects of a threatened plant's management plan. The workflow presented here should help relevant stakeholders design a sampling strategy that directly suits their questions and needs.

1. Introduction

The sequencing and analytical techniques used in conservation genomics have advanced considerably in recent years, making genetic information more accessible to conservation managers (Rossetto and Henry 2014; Williams et al., 2014). In the past, conservation genomic studies have typically been reserved for highly threatened species with well-resourced

* Corresponding author.Research Centre for Ecosystem Resilience, Australian Institute of Botanical Science, The Royal Botanic Garden Sydney, Australia.
E-mail address: maurizio.rossetto@rbgsyd.nsw.gov.au (M. Rossetto).

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recovery programs or were undertaken as part of externally supported long-term research projects. Now, the necessary technical approaches are more widely accessible due to decreasing costs of high-throughput sequencing and the availability of broadly applicable molecular workflows (Supple and Shapiro, 2018).

Much has been written on the current global biodiversity crisis, with threats ranging from habitat loss and fragmentation, to invasive pest species, disease and climate change (e.g., Ceballos et al., 2015). The Australian fires of 2019–2020 provide a sobering example of the increasing impact of combined stressors across natural landscapes (Lindenmayer et al., 2020). While conservation-oriented aims and objectives are very similar for flora and fauna, the logistics of obtaining such knowledge can be noticeably different. Due to their sessile nature, plants are generally easier to sample (Chase and Hills, 1991) and are considerably less stressed or endangered by the collection of tissue material (i.e. a single leaf per individual is often all that is needed). Consequently, conservation genomic studies on threatened plant species can be fast, highly representative and resource efficient (Wee et al., 2019; Exposito-Alonso et al., 2020).

With a thoughtful sampling design, the quality and quantity of the data obtained from a single study can immediately guide multiple aspects of a threatened plant's management plan. Reduced-representation techniques (such as genotype by sequencing) can generate low cost genomic information, and adequate sampling for geographically restricted plant species can usually be achieved efficiently (Rutherford et al., 2019). The resulting genomic information can reveal the evolutionary history of a threatened species which both clarifies taxonomic uncertainty and provides otherwise unattainable insights into its adaptive capacity to respond to the threatening processes that shaped its distribution. These insights are also highly informative during extinction risk assessment evaluations.

Ample evidence suggests that genetic diversity measures directly correlate with phenotypic diversity and population fitness (Ellegren and Galtier, 2016), and that endangered species generally hold considerably less diversity than non-endangered counterparts (Spielman et al., 2004). As a result, describing the distribution of the remaining diversity, is critical to the long-term *in situ* survival of threatened taxa as well as to the establishment of representative *ex situ* collections and of translocated populations (Commander et al., 2018; Bragg et al., 2020).

The target audience of this work are biodiversity managers and conservation biologists, and our aim is not to describe in detail the potential and objectives of conservation genetic/genomic studies (Frankham et al., 2002; Funk et al., 2019), nor to present decision trees for potential genetic-based conservation strategies (Ottewell et al., 2016). Rather, we present a simple, standardized workflow for genomic research that has been successfully used to guide the design of management strategies across disparate threatened plants (Rutherford et al., 2019; Bragg et al., 2020, 2021). The workflow is directly aimed at supporting practical outcomes, with relevant management questions forming the basis of sampling and interpretational strategies. It is conceivable that an ever-improving collection of resource-efficient genomic studies could be used to enhance the effectiveness and efficiency of recovery programs for many, if not the majority of, threatened plants (Weeks et al., 2011; Exposito-Alonso et al., 2020). A comprehensive uptake would amplify resource efficiencies, enable cross-species comparisons and potentially allow for the development of evidence-based broad-scale generalisation in conservation practices.

The key to a well-designed study that can maximize practical benefits is to get the sampling right. A single run of genotyping by sequencing can have multiple applications, resulting in a 'one cost with many benefits' outcome. We provide an overview of how to design a simple and cost-effective sampling strategy, identify the main questions the study can answer and, using case studies, provide insights into how genomic information can directly guide applied recovery actions.

2. A standardized workflow: from sampling to applied management actions

The key to a well-designed project is a sampling strategy that can help address targeted questions (Hartl, 2020). A conservation genomic study should be completed 'up-front and early' during the development of a threatened plant's recovery program, with a clear understanding of the questions that the sampling and analyses will need to address in order to facilitate recovery. Once all the relevant permits have been obtained, consideration should be given to sampling opportunities that will maximize limited resources. Given the relative ease with which samples for genomic studies can be collected (recognizing that plant location, habit and identification can sometimes hinder progress), sampling can be efficiently incorporated into initial targeted surveys or subsequent monitoring inspections (e.g. when seasonality influences our perception of a species' distribution).

During sampling, it is critical to collect all relevant metadata (e.g. including ecological information such as phenological state, size of population, habitat condition, distribution, *etc*), and ascribe a unique identifier (UID) that will follow the individual plant throughout the analyses, interpretations and dedicated management actions. Collection apps designed for the sampling of genetic material can help ensure that the appropriate information is easily collected and transferred to follow-on analytical interpretations, databases and on-ground activities (Rossetto et al., 2019).

An overview of the design for an effective conservation genomic workflow is illustrated in Fig. 1. It includes a sampling strategy that can be adjusted according to the major issues likely to affect the recovery process, and highlights how the analyses derived from the single comprehensive sampling event can be interpreted and directly applied to recovery actions. Although we take into consideration the major issues usually faced by threatened plant species, we recognize that there will be instances where modifications might be needed.

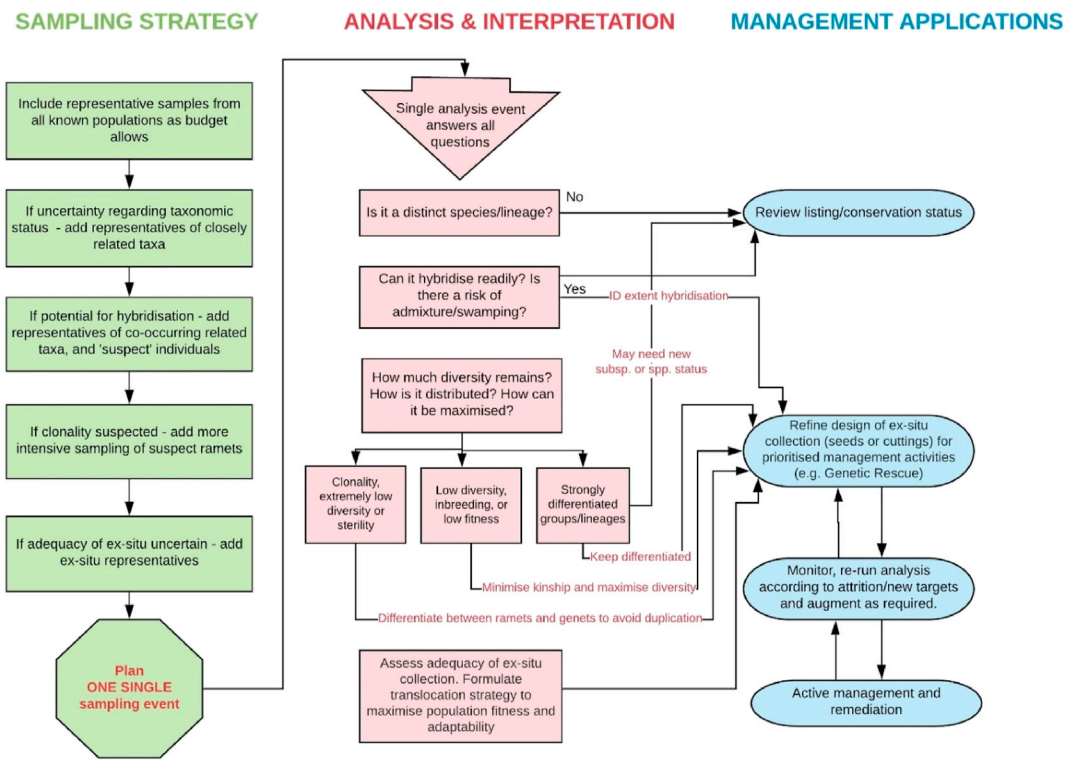

Fig. 1. A simple design for an effective conservation genomic workflow. An overview of a workflow for conservation genomic studies of threatened plant taxa. The sampling needed for a set of universal questions that a genomic study may address is listed on the left, along with the type of analytical interpretation that can be derived and directly applied to on-ground management strategies including restoration, monitoring and follow-up adaptive management.

2.1. Sampling strategy

Knowing how much genetic variation remains within and among populations has important implications for the management of threatened species. The level of genetic diversity within populations influences its reproductive output and resilience, and the distribution of diversity among populations guides decisions regarding population prioritization and whether to avoid or facilitate mixing differently sourced individuals during *in situ* and *ex situ* management activities (Frankham et al., 2017; Ralls et al., 2020). To delineate the spread and dynamics of the remaining genetic diversity, threatened plants that are geographically restricted can be representatively sampled across their entire distribution (in extreme cases including every known individual; Rossetto and Kooyman, 2005). For more widespread taxa, sampling entire populations may not be practical or within the financial scope of the study (see Case Study 2), and in such circumstances sampling should be representative of the specific objectives identified within the recovery plan. Below we identify a range of circumstances that might lead to slight adjustments in the sampling strategy (Fig. 1).

Some threatened species might have been described based on minor morphological and/or distributional features, without consideration for evolutionary history and distinctiveness. Such information is not always adequate to ascertain taxonomic boundaries, and if doubt remains about species recognition, then validation is a necessary first step (Naciri and Linder, 2015). For an evaluation of taxonomic status to be possible, adequate sampling is needed from closely related taxa sourced from sympatric or sufficiently proximate individuals where possible, or from good quality (i.e., recently collected and carefully preserved) herbarium specimens. The inclusion of such a relatively small additional sample set, can go a long way to provide the necessary evolutionary context and establish if lineage differentiation is justified. These same individuals (and representative herbarium vouchers) could also be used in associated phenetic studies, allowing for a robust comparison between genotype and phenotypic variation.

While hybridization may be a natural and important evolutionary process in plants (Rieseberg et al., 2003; Hegarty and Hiscock, 2005), understanding the extent of hybridization is critical to threatened species' management as it can impact on conservation values and operational decision-making (Jackiw et al., 2015). Failing to appreciate the occurrence and scale of admixture can compromise the purity of remaining populations and impact on translocation plans (Rutherford et al., 2019; Winkler and Massatti, 2020). Observation-based means of detecting hybrids (i.e. based on intermediate morphological characters) can overlook cryptic hybrids and assign them erroneously to a species (Rutherford et al., 2018, 2019). Consequently, if there is potential for hybridization to occur *in situ* or *ex situ*, genetic samples and representative vouchers should be

collected from sympatric congeneric taxa to facilitate comparative analyses (where the two species co-occur as well as from disjunct sites as controls). Sampling can broadly replicate the sampling strategy used for taxonomic ascertainment, with the inclusion of phenotypically intermediate individuals and seeds from 'suspect' individuals.

Extensive clonal reproduction (for example via stem fragmentation, stolons, rhizomes, or tubers) can cause a disconnect between ramets (genetically identical stems) and genets (genetically distinct individuals), and thereby confound population size estimates (Bond and Midgley, 2001). In cases where clonality is suspected, more intensive localized sampling (at set distances relevant to the population size) should be performed in order to confirm vegetative growth and identify patterns that could help distinguish ramets from genets. Recognizing that a target species is potentially much rarer than initially forecasted is critical, as populations of hundreds of ramets can sometimes represent single genets (Lynch et al., 1998; Rossetto et al., 2004) prompting a reassessment of threat status.

Understanding a species' mating system through the addition of progeny arrays, can also provide valuable management information. However, collecting seeds can add complex temporal constraints to the sampling strategy, as well as increase technical requirements (e.g., seed storage and germination) and costs involved (Guja et al., 2015). As a result, such studies are usually considered as more suited for an eventual second round of knowledge gathering.

Finally, all the factors that impact on the viability of wild populations, are also relevant to existing and planned *ex situ* collections. The strategy for selecting representative individuals held within *ex situ* collections can vary and the resulting assortment of genotypes is unlikely to be directly useable in conservation-related activities without careful planning and the availability of relevant genetic information (Commander et al., 2018).

2.2. Analytical interpretations and practical applications

A single, practically designed sampling strategy will provide the data necessary for multiple interpretative analyses that will directly inform applied management approaches and clarify issues raised within species-specific recovery plans (Fig. 1). Some illustrative examples are explained below.

If genomic evidence does not support preliminary taxonomic status, the outcome is likely to impact significantly on management and on the deployment of the limited resources available. While the conservation implications of cryptic species have been extensively reported in the scientific literature (e.g., Bickford et al., 2007), the reassessment of taxonomic status for a previously described threatened species is less common. Our Case Study 1 ('*Banksia vincentia*') demonstrates how starting the management process from a genetic study with the aim of clarifying species status could have prevented listing the population at Vincentia as Critically Endangered, and averted the use of limited human and financial resources.

Hybridization and introgression can significantly impact *in situ* and *ex situ* management strategies. Environmental degradation processes that diminish habitat availability and cause population declines, can facilitate stochastic hybridization events, and/or the formation of more established hybrid zones (McIntosh et al., 2014). Admixture increases where small populations are surrounded by individuals from closely related taxa causing elevated inter-specific gene flow into an already bottlenecked population. This can lead to genetic swamping, where a preference for outcrossed pollination increases the uptake of pollen from other species (Levin et al., 1996; Bohling, 2016) resulting in genetic assimilation by the more frequently occurring taxon (Todesco et al., 2016). Understanding the risks associated with hybridization has significant consequences on the selection of individuals and locations to be used in translocation projects and in *ex situ* collections (as represented by living propagules and/or seed banks).

Similarly, while polyploidy is also a relatively common evolutionary process potentially leading to speciation in plants (Van de Peer et al., 2009), recognizing the natural distributional and reproductive boundaries between various ploidies can provide critical guidance to *in situ* and *ex situ* management strategies (Pickup and Young, 2008). Although the accurate detection of ploidy variation can be complex, a growing array of dedicated population genetic tools makes the detection of landscape-level patterns increasingly tractable (Ahrens et al., 2020).

Maximizing genetic diversity through genetic rescue and other strategies is a major component of threatened species recovery (Bell et al., 2019). Within that context, an understanding of mating systems can impact diversity-related decisions. While species that are capable of selfing might be less prone to inbreeding depression, they might also be more susceptible to drift and have limited adaptive potential (Hartfield, 2016). At the extreme end of low genetic diversity, clonal populations comprising small numbers of genets can have reduced fertility (especially if they are preferential outcrossers; Scobie and Wilcock, 2009) and diminished resilience to stressors such as climate change, disease and competition. Similarly, the inadvertent over- or under-contribution from single genets to *ex situ* collections, can result in translocated populations that do not maximize the limited available evolutionary potential of the target species (Greenfield et al., 2016). Within such circumstances, the additional information provided by the estimation of pairwise similarity or kinship can facilitate the identification of clones, differentiate them from closely related genets and guide improved translocation strategies (Bragg et al., 2020).

Translocations and other recovery-related activities are common objectives justifying the development of *ex situ* collection. Genetic rescue and the maximization of genetic diversity in managed populations can increase their fitness and adaptive resilience (Frankham et al., 2002; Bell et al., 2019), however it is important to remember that randomly increasing the number of represented individuals alone is unlikely to achieve such goals. To avoid unexpected shortfalls in long-term diversity targets, a range of genome-based approaches can be employed.

First, genetic data can help determine the size of the representative population (comprising living propagules or seeds) that is needed to capture a target proportion of diversity as identified in reference populations (Marshall and Brown, 1975). Usually, as more individuals are incorporated into a collection of plants, allelic diversity (as a measure of diversity) increases as a function of population size until saturation (when most common alleles in the reference population are sufficiently represented; Richards et al., 2007; Griffith and Husby, 2010; Hoban et al., 2020b). As artificial populations can be expected to lose alleles through casualties (Richards et al., 2010), the proportion of alleles targeted for founding populations should be ambitious.

Second, genetic data can help adjust the composition of a representative population to promote genetic diversity while reducing inbreeding and drift. Where inbreeding depression is of concern, it is possible to choose a population where the individuals have low levels of average pairwise relatedness (Bragg et al., 2020). Choosing individuals that are unrelated or that are observed to be genetically diverse, can capture large numbers of alleles from a smaller sample and promote high diversity in re-established populations (e.g., Marshall and Brown, 1975; Schoen and Brown, 1993; Richards et al., 2007). This is important where there are substantial constraints on the number of genotypes that can be included in an *ex situ* collection or a translocated population, as avoiding the inclusion of genetically similar individuals (such as close relatives) constrains the overrepresentation of alleles that are identical by descent. A possible approach (Bragg et al., 2021) is to genotype many individuals and examine the genetic diversity of different candidate collections that vary in both their size and composition. This makes it possible to simultaneously contemplate the effects of population size (e.g., $N = 20, 24, 28, 32, 36, 40$) and composition on genetic diversity.

Third, the progeny from a living germplasm collection might be used in conservation plantings (a seed orchard, or seed production area). To promote diversity within the progeny produced by such a collection, genetic data can be used to arrange plantings in ways that reduce the likelihood that related individuals will breed with each other (Kashimshetty et al., 2012). This might be particularly useful for endangered species with small source populations, where related individuals will be used in a seed orchard (Bragg et al., 2021).

3. Study cases of the 'one cost, many benefits' workflow

To demonstrate the use of the workflow, we briefly summarise two case studies. These two projects involve threatened plants with very different conservation circumstances and management needs, where similar sampling strategies and analytical approaches resulted in contrasting conservation outcomes. Additional details about the sampling strategy, the analyses used, and the findings can be found in Appendix A (Supplementary Material).

3.1. Case Study 1: 'Banksia vincentia' – taxonomic misidentification leads to unnecessary actions

Banksia vincentia M.L. Stimpson & P.H.Weston was recently described as part of a species complex that includes *B. collina* R. Br., *B. cunninghamii* Sieber ex Rchb., *B. neoanglica* (A.S.George) Stimpson & J.J.Bruhl and *B. spinulosa* Sm. (PlantNet; https://plantnet.rbgsyd.nsw.gov.au/). As it was identified from a single population within a 100 m^2 area at Vincentia New South Wales (NSW, Australia) consisting of nine individuals (five of which are immature saplings), '*B. vincentia*' is currently listed as Critically Endangered in NSW. A phenetic analysis identified '*B. vincentia*' as sharing similarities with *B. neoanglica* (Stimpson et al., 2014) but, so far, no cladistic analyses have been used to test a monophyletic species concept for '*B. vincentia*' or the other entities in the species complex.

Due to the extreme circumstances that included a fire and an unexplained progressive decline of '*B. vincentia*', *ex situ* propagation and translocation activities were initiated in the absence of the genetic evidence needed in support of a rigorous test of taxonomic status. Considering the need of prioritising the conservation resources available, a study was eventually initiated to assess species status, genetic diversity (qualitatively and quantitatively), genetic health and hybridization risk and extent. To achieve these objectives, we sampled all known individuals of '*B. vincentia*' (including a representative sample from *ex situ* collections) and multiple representative from the relevant species complex.

Is taxonomic status confirmed? Nuclear genomic data sampled across the full extent of the species complex (*B. collina*, *B. spinulosa, B. cunninghamii*, *B. neoanglica, 'B. vincentia'*) did not support the description of '*B. vincentia*' as a distinct species (Fig. 2). Genomic analyses show that all samples of '*B. vincentia*' (excluding hybrids which are discussed below) are nested within *B. neoanglica*, and the individuals at Vincentia are assigned to *B. neoanglica* populations in the Boonoo Boonoo National Park (NP) and Girraween NP area (Fig. 2). Such strong assignment conflicts with latitudinal differentiation across well-recognized biogeographic barriers, between-group admixture detected across the barriers, and a broader isolation-by-distance pattern (i.e. correlation between genetic and geographic distances; $R = 0.719$, $p < 0.001$) detected across the whole species complex (Fig. 2). Thus, the genomic data indicate that this is not a distinct species and that the current distribution is more likely a result of an historical planting. Although this might circumvent the need for additional investigative analyses, we briefly summarise additional outcomes since these were part of the workflow.

Is hybridization a risk? The population at Vincentia is surrounded by three other common *Banksia* species, *B. ericifolia* L. f., *B. paludosa* R. Br., and *B. spinulosa*. A previous phenetic study could not detect evidence of hybridization among 'contact zones' within the *B. spinulosa* species complex (Stimpson et al., 2014). However, genomic data found evidence of hybridization with both *B. spinulosa* and *B. ericifolia* (Fig. S1), including a *B. spinulosa* x '*B. vincentia*' hybrid (NSW1033079) that had been morphologically determined as *B. spinulosa*.

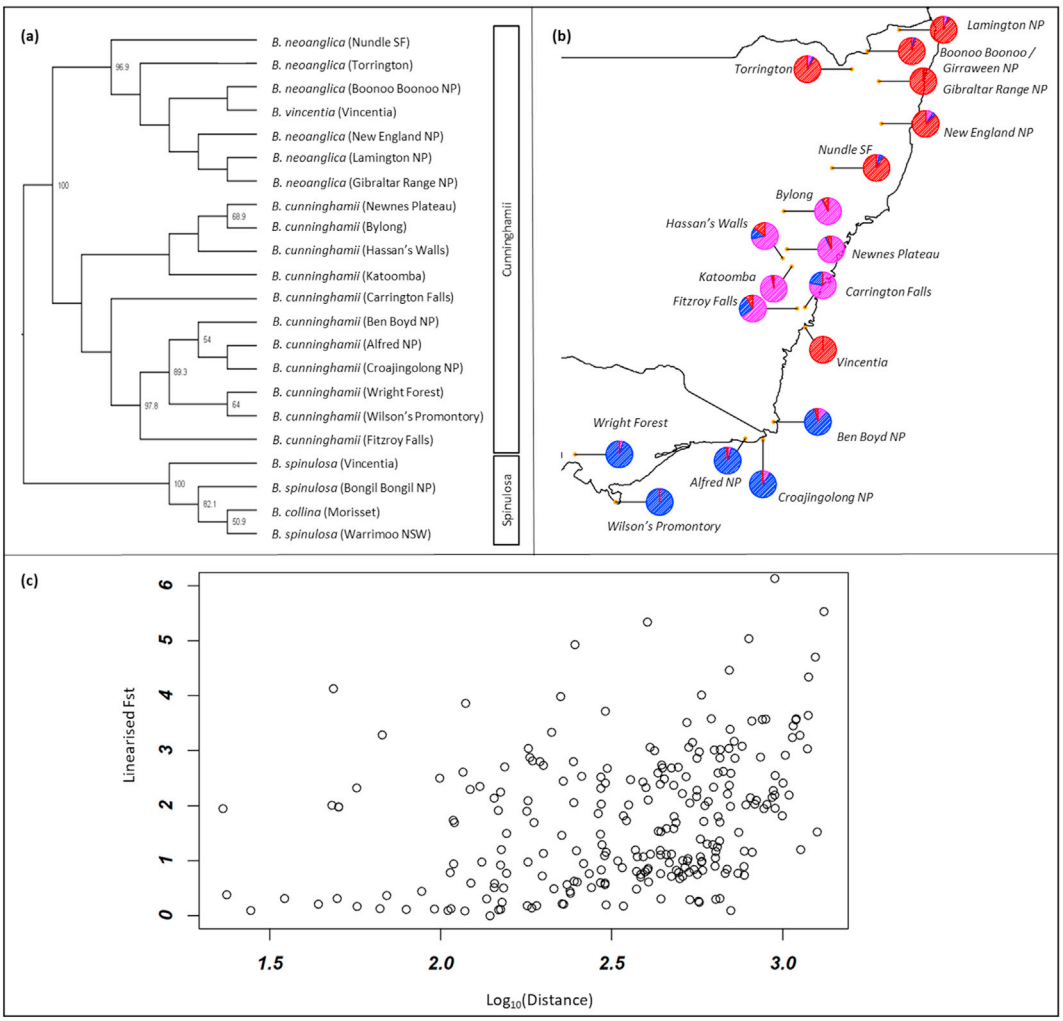

Fig. 2. Applying genetic analysis and interpretation to investigate the validity of a distinct species concept. (a) SVD Quartets coalescent analysis with bootstrap support values above 50% are placed at the nodes associated with relevant branches. Total weight of incompatible quartets = 1556 (21.22%), and total weight of compatible quartets = 5759 (78.72%). (b) Nuclear DNA-based population structure output based on averaged ancestral assignment of K = 3 across populations of the '*B. vincentia*'-*B. neoanglica*-*B. cunninghamii* clade in New South Wales and Victoria (Australia). (c) Isolation by distance graph for individuals from the '*B. vincentia*'-*B. neoanglica*-*B. cunninghamii* clade. F_{ST} values increase corresponding with distance (meters) between any two given individuals, demonstrating the trend of isolation by distance. Results from a Mantel test using 9999 permutations provided a statistically significant ($p < 0.001$) R value of 0.719. Untransformed datasets provided an R value of 0.452 ($p < 0.001$).

Are *ex situ* collections adequate? The hybrid status of several plants propagated from seeds at the Australian Botanic Garden Mount Annan had long been suspected since many are morphologically distinct from the plants at Vincentia and share morphological similarities to *B. ericifolia*. Nuclear DNA analyses recovered 17 hybrid plants.

How much diversity is there and is there clonality? Although no clonality was detected, of the nine remaining individuals that were successfully sampled at the Vincentia site, five individuals (G007a–d) were found to be closely related (Fig. S2). An additional mature individual (G013) grouped closely to G007d (i.e. likely paternal parent), resulting in high overall kinship values.

3.2. Case Study 2: Daphnandra johnsonii, the workflow informs simple practical decisions across a complex matrix of issues

Daphnandra johnsonii Schodde (Atherospermataceae), commonly known as the Illawarra Socketwood, is a medium sized rainforest tree principally found in disturbed remnants, rocky sites and gullies in the foothills of the Illawarra Escarpment (New South Wales, Australia). It has bisexual flowers and produces achenes likely to be wind dispersed, but often attacked by gall midges (Kolesik et al., 2019). Although poor reproductive success has been recorded across most populations, the species can persist locally through prolific coppicing (producing multiple branches at stem base) and suckering (resprouting away from the stem from rhizomes).

A conservation genomic study following the workflow described here, aimed at developing effective management strategies for *D. johnsonii* that would ensure long-term viability. A total of 188 individuals were analysed with sampling focused on 16 geographically representative sites (Fig. S3), selected based on available resources and on the fact that some populations had restricted access.

Is taxonomic status confirmed? Until a recent taxonomic revision of the genus (Foreman and Whiffin, 2007), *D. johnsonii* was described as *Daphnandra* sp. C "Illawarra", and before this was included in the morphologically similar *D. micrantha* (Tul.) Benth *s. lat*. Consequently, in order to confirm the distinctiveness between these two species, representative individuals from all regional members of the genus were analysed: *D. micrantha* (N = 12), *D. tenuipes* (N = 6) and *D. apetala* (N = 6). Splitstree analysis, Principal Component Analysis (PCA, Fig. 3) and phylogenetic analysis (Fig. S4) of the SNP dataset confirmed the distinct taxonomic status of *D. johnsonii*. *Daphnandra johnsonii* individuals formed a well-supported clade sister to *D. micrantha*, with *D. apatela* and *D. tenuipes* diverging earlier on the tree.

Is hybridization a risk? The potential for hybridization within this species, genus or family was yet to be determined and was not expected to be an issue. Although currently *D. johnsonii* is not sympatric with congeneric species, the genomic data identified the likely signal of historical admixture. Both Splitstree and PCA revealed that three samples from the Broughton Village site did not cluster with the other *D. johnsonii* individuals, but instead had an intermediate position and displayed reticulation with *D. micrantha* (Fig. 3). This identifies a likely, previously undetected historical hybridization event between these two species.

What is the remaining diversity and is it clonal? Despite the apparent reliance on coppicing and resprouting, the extent of clonality within *D. johnsonii* was yet to be evaluated (NSW Department of Environment and Conservation, 2005). While some populations comprise a single stem (ramet), others span over several hundred metres comprising an unknown number of genets (genetically distinct individuals). Table 1 lists the number of individuals sampled and the number of genets present at each site. Each *D. johnsonii* site consists of at least one unique genet (with no single genets being replicated at more than one site), but the extent of clonality varies from site to site. Some clones extend across a considerable distance, as found at the

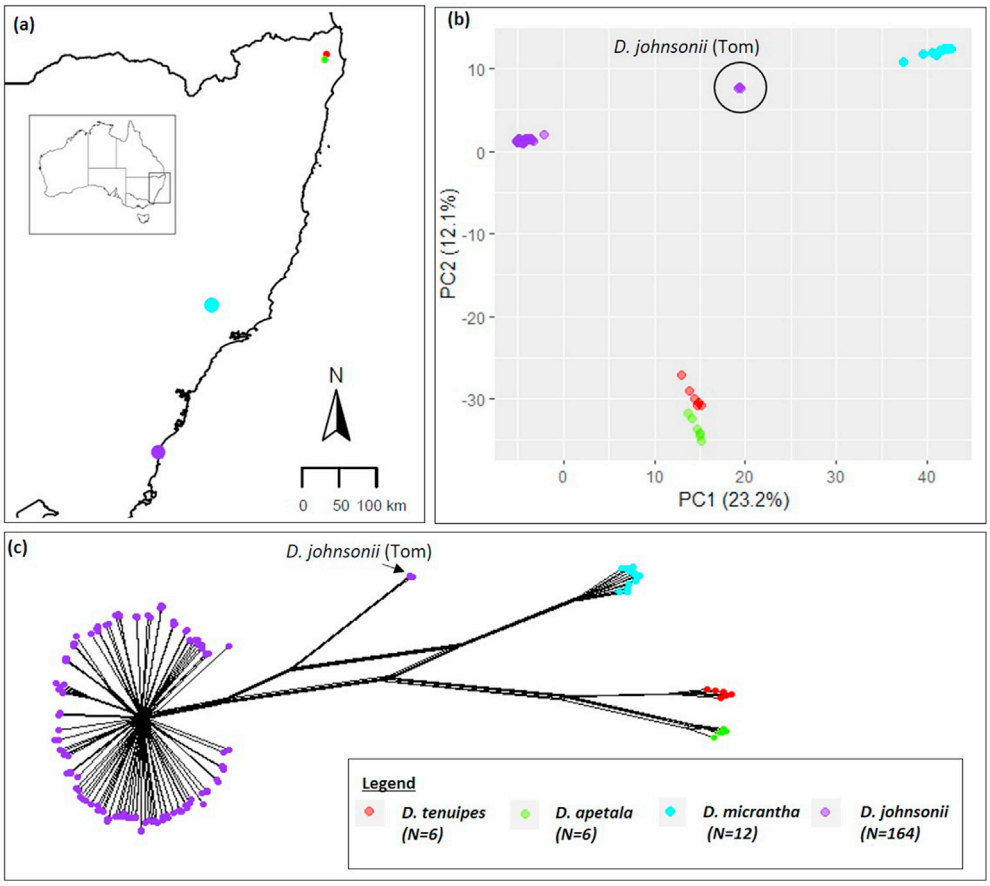

Fig. 3. Applying genetic analysis and interpretation to investigate species taxonomy. (a) Sampling locations along the east coast of New South Wales, Australia; (b) Principal Component Analysis (PCA); (c) Splitstree network analysis (c) of SNP data from individuals from four *Daphnandra* species: *D. tenuipes*, *D. micrantha*, *D. apetala* and *D. johnsonii*. Splitstree and PCA identified three samples (collectively labelled as "*D. johnsonii* (Tom)" from Broughton Village) located in an intermediate position between *D. johnsonii* and *D. micrantha*.

Table 1
Diversity derived from single nucleotide polymorphism (SNP) data for populations of *Daphnandra johnsonii* with one or more genets (i.e. after the removal of clonal individuals). Table lists allelic richness (Ar), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}). N_R represents the number of ramets sampled and N_G the number of genets identified at each site. Values in brackets represent standard deviations. F_{IS} was not measured at Avon, Stoney or Tom sites as each had only one genet. The "WBG" site contains unique genets observed in the *ex situ* collection at Wollongong Botanic Garden.

Site	N_R	N_G	Ar	H_O	H_E	F_{IS}
Avon	6	1	1.282 (NA)	0.271 (NA)	0.142 (NA)	NA
Bayden	11	2	1.402 (0.003)	0.268 (0.003)	0.193 (0.001)	−0.351 (0.006)
Bvale	7	4	1.485 (0.001)	0.253 (0.002)	0.249 (0.001)	0.011 (0.006)
Curra	10	4	1.442 (0.004)	0.270 (0.003)	0.224 (0.002)	−0.057 (0.006)
Fount	7	3	1.444 (0.002)	0.257 (0.001)	0.221 (0.002)	−0.062 (0.006)
Fox	7	3	1.471 (0.002)	0.270 (0.002)	0.233 (0.001)	−0.101 (0.005)
Jerr	6	3	1.491 (0.004)	0.291 (0.002)	0.247 (0.002)	−0.129 (0.008)
Marsh	10	2	1.322 (0.003)	0.270 (0.002)	0.161 (0.002)	−0.444 (0.005)
Minnamurra	16	7	1.560 (0.001)	0.257 (0.001)	0.285 (0.001)	0.094 (0.002)
Rose	8	5	1.568 (0.001)	0.276 (0.002)	0.285 (0.001)	0.029 (0.006)
SOS	10	7	1.540 (0.003)	0.265 (0.002)	0.267 (0.002)	−0.001 (0.002)
Stoney	6	1	1.263 (NA)	0.250 (NA)	0.133 (NA)	NA
Tom	6	1	1.276 (NA)	0.268 (NA)	0.138 (NA)	NA
Tooli	24	18	1.635 (0.003)	0.269 (0.002)	0.320 (0.001)	0.150 (0.002)
WBG	12	7	1.505 (0.002)	0.206 (0.001)	0.278 (0.001)	0.268 (0.003)
Whisp	6	4	1.539 (0.002)	0.274 (0.001)	0.273 (0.001)	0.003 (0.003)
Willow	12	2	1.494 (0.002)	0.287 (0.001)	0.243 (0.002)	−0.193 (0.006)

Curra site where one widespread genet has ramets distributed across a transect of over 900 m (Fig. 4). This shows that the location of single stems is not sufficiently informative to identify clonality, estimate overall diversity, and prevent duplication of genetic material in *ex situ* collections.

Genetic diversity across all sites is summarised in Table 1, and as for most threatened species with small populations and limited geographic distribution (Spielman et al., 2004), *D. johnsonii* retains relatively low levels of allelic diversity and heterozygozity. Most sites displayed similarly moderate levels of heterozygozities and the number of genets did not influence the level of heterozygozity as calculated after the exclusion of multiple clonal replicates (e.g. Tooli has the highest number of genets but not the highest observed heterozygozity). Most populations displayed negligible levels of inbreeding (Table 1) suggesting that this species is most likely a preferential outcrosser. Across the whole species, pairwise F_{ST} measures support an isolation by distance pattern, where gene flow decreases among *D. johnsonii* sites the further these are distributed from each other (Fig. S5), without strong structure or differentiation among geographical areas (Fig. S3).

Are *ex situ* collections adequate? Kinship analysis matched the source of all but three *ex situ* collection plants at the Wollongong Botanic Garden (WBG). One sample from WBG (NSW1045870) was not related to any of the *D. johnsonii* individuals sampled with the UPGMA tree suggesting closeness to Bayden (Fig. 4). Another two samples (NSW1027850, NSW1027855) are also genetically distinct from other sampled *D. johnsonii* individuals and are most genetically similar to individuals from the Marsh site. The overall results suggest the *ex situ* collection are unrepresentative for a translocation program, although they also contain individuals that might originate from unsampled sites.

Can we develop a suitable translocation plan? We used genomic data to estimate the necessary combinations of propagules that will ensure the establishment of evolutionary resilient translocated populations of various sizes (Fig. S6). Propagation populations were designed by examining the proportion of common alleles in *D. johnsonii* to be included in each propagation population. Propagation populations that were optimised on the basis of genomic data captured more SNPs than random ones (Fig. S7), with a translocation population targeting 20 diverse genets (listed in Fig. S6) being able to capture more than 96% of all common alleles (Fig. S7).

4. Conclusions

4.1. Interpreting and applying the knowledge gained via the workflow

The two case studies show how a standardized knowledge-gathering workflow can be applied in very different circumstances and lead to considerably different management outcomes. The first example provides a simple outcome with very direct consequences: no support for distinct species status thus no need for management actions (although unfortunately some activities had already been initiated). The second example produces more complex (and somewhat unexpected) interpretations, but also conceptually simple management solutions.

'Banksia vincentia' is not a distinct species. Rather it is part of a northern population group of *B. neoanglica* and is most closely related to the population found at Girraween NP and Boonoo Boonoo NP. This similarity can be explained by two possible interpretations: 1) the population at Vincentia is a recently disjunct remnant of previously more widespread clades that are now restricted to north of the Hunter River Corridor (i.e. populations currently referred to as *B. neoanglica*); 2) the Vincentia population has been recently transported to the site from a northern New South Wales source. Although the second

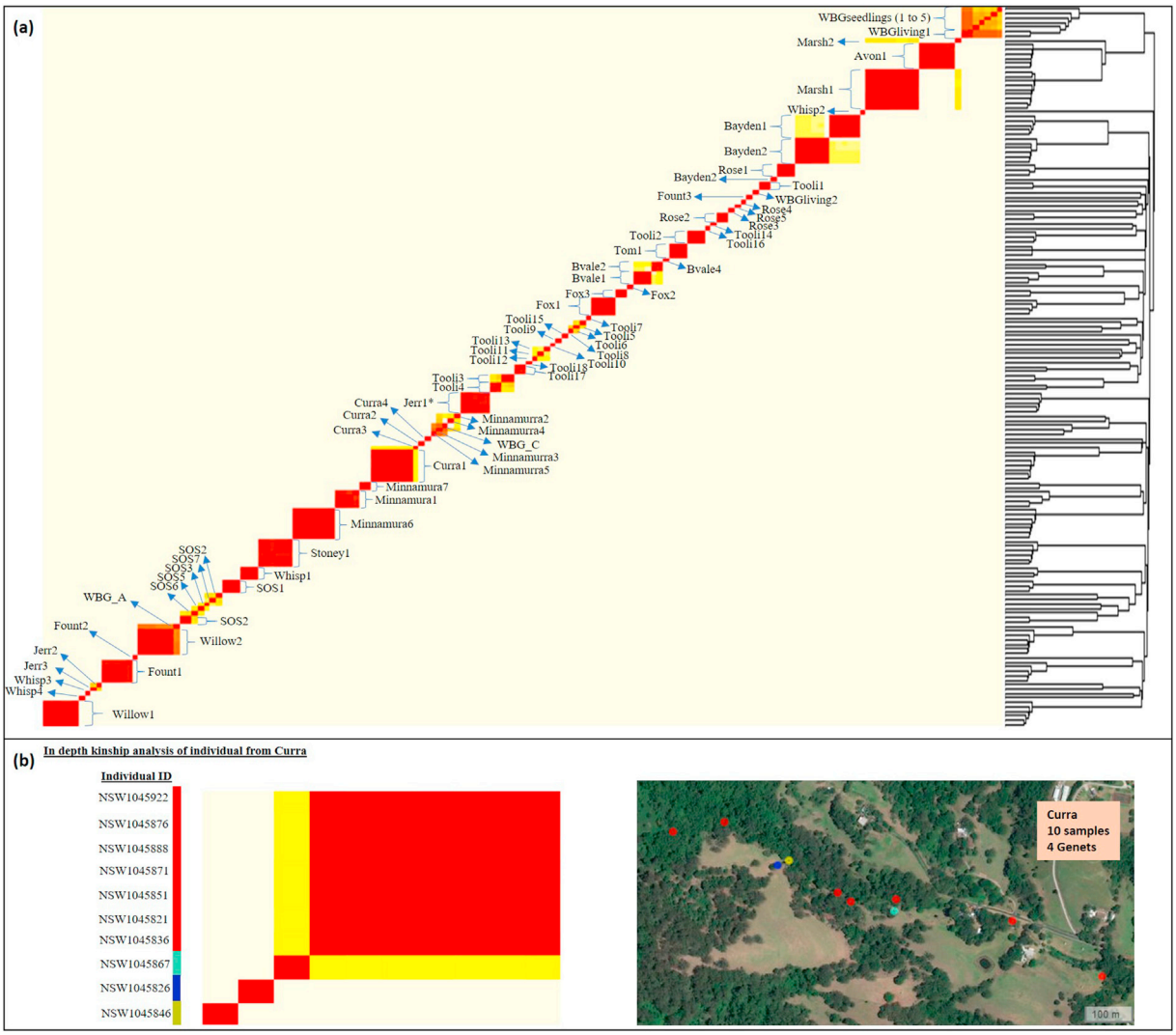

Fig. 4. Kinship, relatedness and clonality in *Daphnandra johnsonii*. (a) Kinship or relatedness heatmap/composite UPGMA tree derived from the SNP data for 161 *in situ* and *ex situ* specimens of the *Daphnandra johnsonii*. The heatmap displays kinship estimated for each pair of samples for each site, (see Fig. S2 for site location), red coloration corresponding to the highest kinship (0.44 or greater = clone), orange-yellow coloration corresponding to medium pairwise kinship coefficients (less than 0.4 but greater than 0.25 = sibling) and white corresponding to the lowest kinship (0). The descending red diagonal on the graph is therefore the result of an individual matched with itself. The 161 specimens exclude the three individuals from Tom (Broughton Village). (b) Kinship heatmap (left) and location map of individuals at Curra. Kinship is measured for each individual and its geographic position is traced back to the location map to determine if multiple ramets of a genet are situated in proximity of each other. Multiple representatives of a single genet are assigned the same color, with different genets being assigned different colors. The results indicate that for Curra, the seven ramets belonging to the same genet stretch over 900 m, with other genets being interspersed within them. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

option may initially sound improbable, the genomic evidence clearly assigns the individuals at Vincentia to the *B. neoanglica* population at Boonoo Boonoo, showing no gene flow with surrounding populations as expected from the genetic and geographic gradation found across the broader species complex. As the empirical analyses do not recognize *'Banksia vincentia'* as a distinct species, reassessment of its threatened status will be necessary. Additionally, extensive hybridization was detected at the Vincentia site and amongst the *ex situ* collections, further emphasizing that caution is required when assuming a 'pure' genotype through evaluation of morphological characters for species with unsubstantiated taxonomic origins.

Daphnandra johnsonii was confirmed as a distinct species in need of active management, and the sampling strategy facilitated a rapid appraisal of what natural diversity remains, and how it should be managed. The genetic signature of past hybridization events was unexpectedly identified at one site, suggesting that cross-specific pollen uptake driven by low genetic diversity can leave long-lasting effects for *D. johnsonii*. Ancient hybridization events can still be revealed within long-lived clonal populations (Rossetto et al., 1997), and although there are no sympatric occurrences of *D. micrantha,* the genus

Daphnandra is likely to have been historically more widespread like other related genera within Atherospermataceae (e.g. *Atherosperma*, Worth et al., 2011). While admixture may be a natural and relatively common process and current circumstances do not highlight immediate threats, caution needs to be taken in avoiding potential overlaps with congeneric taxa during translocation practices.

More importantly, the extensive clonality detected for *D. johnsonii* is likely to contribute to the recorded loss of fertility (as previously documented among other rare rainforest trees; Rossetto et al., 2004; Eliot et al., 2015) and its susceptibility to pests and disease (Kolesik et al., 2019). With preference for outcrossed pollen, population-level isolation and high levels of clonality, most populations are effectively sterile and potentially less resilient to climatic changes and other stressors. Therefore, as the effective population size of *D. johnsonii* is much smaller and less resilient than initially thought, active management actions such as translocation and/or augmentation are warranted. Given that current *ex situ* collections for *D. johnsonii* are unrepresentative and contain repeat collections from the same genet, they cannot be used as the sole source for translocation or other management actions. Genetically guided management actions will facilitate the establishment of novel mating combinations to boost overall fitness and increase long-term viability (Fig. S6).

4.2. A standardized workflow to inform and guide practical management actions for threatened species

Genomic datasets have become faster and cheaper to generate, and at the same time offer greater power to inform management decisions (Luikart et al., 2018). As such, we should shift from asking whether to undertake a genomic study of a threatened species, to asking how to undertake it as effectively and efficiently as possible. More importantly sampling and interpretational strategies should be guided by management-oriented applications from the onset (Hohenlohe et al., 2020). Here we propose a standardized workflow that can inform and guide the direct application of genomic information to the management of threatened plants. The flowchart in Fig. 1 guides stakeholders responsible for the development of management actions with designing a suitable sampling strategy, while gaining an understanding of how the genetic knowledge will directly answer their questions and support their recovery efforts. The workflow emphasises how a single, well-designed and standardized study can be applied to multiple species and scenarios to answer a suite of critical questions, even when limited background knowledge is available.

A key to the success of this workflow is to secure a sampling strategy that can achieve multiple analytical interpretations and directly support conservation activities without preventing or delaying other threat-mitigation operations. Such knowledge infrastructure can also help define recovery success, identify relevant milestones and plan long-term monitoring (Van Rossum et al., 2020). There is significant scope to refine genetic monitoring strategies in the context threatened species' management, with baseline data standardization and accessibility being critical to long-term uptake, and integration within Convention of Biological Diversity targets (Hoban et al., 2020a). More importantly, as conservation genomics can drastically change the direction of a recovery program (e.g. Case Study 1), relevant analyses should be considered as part of the routine first steps in status listing, and in the development of a recovery plan for threatened species.

The workflow does not intend to be an exhaustive exploration of all biological and evolutionary contexts that are relevant to threatened species, but to simply and efficiently resolve major aspects of threatened species management and conservation. As available tools evolve rapidly, so will the quantity and quality of gathered knowledge and the range of management interpretations provided (Browning and Browning, 2015). For instance, the increasing availability of reference genomes is providing new opportunities for data gathering (Holliday et al., 2018), as well as for bioinformatic, analytical and interpretational developments (Salojärvi, 2018), and the detection and management of true adaptive variation across remnant populations (Funk et al., 2019). New open-access, collaborative programs such as the Threatened Species Initiative (https://threatenedspeciesinitiative.com/) and the Zoonomia project (Zoonomia Consortium and Casewell, 2020; https://zoonomiaproject.org/) are at the forefront of these new developments.

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Data availability statement

The code used to filter genetic data and implement the analyses described above is available in a Mendeley Data repository (https://doi.org/10.17632/g5hhnd5bc9.1).

Declaration of competing interest

All authors confirm that there is no conflict of interest in the execution of this study or the submission of it here to Global Ecology and Conservation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2021.e01492.

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